Cell Stem Cell, volume *11*

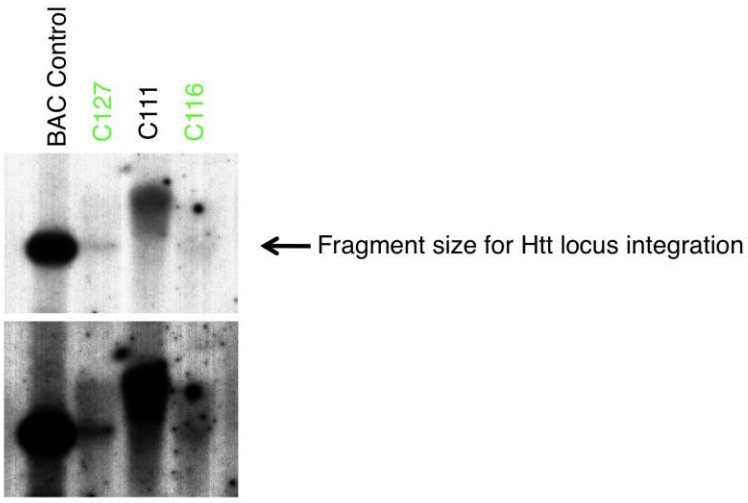
Supplemental Information

Genetic Correction of Huntington's Disease Phenotypes in Induced Pluripotent Stem Cells

Mahru C. An, Ningzhe Zhang, Gary Scott, Daniel Montoro, Tobias Wittkop, Sean Mooney, Simon Melov, Lisa M. Ellerby

Figure S1

A

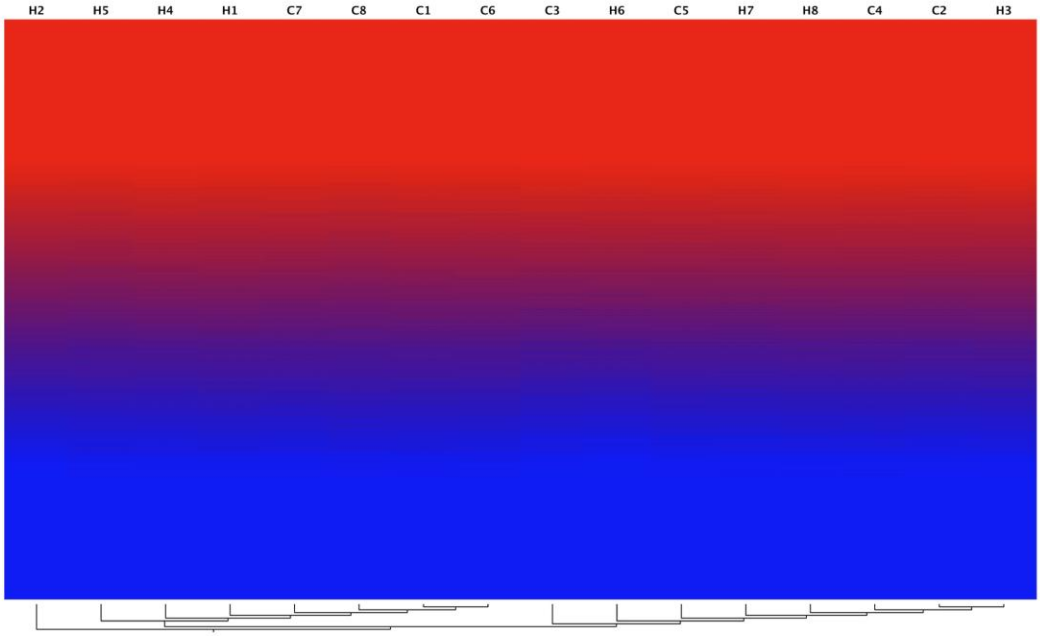


B

```
CLUSTAL 2.1 multiple sequence alignment

HDiPS_Short       TACAGTCTCACCACGCCCCGTCCCCTCTCCGTTGAGCCCCGCGCCTTCGCCCGGGTGGGG 60
HDiPS_Long        TACAGTCTCACCACGCCCCGTCCCCTCTCCGTTGAGCCCCGCGCCTTCGCCCGGGTGGGG 60
C127              TACAGTCTCACCACGCCCCGTCCCCTCTCCGTTGAGCCCCGCGCCTTCGCCCGGGTGGGG 60
C116              TACAGTCTCACCACGCCCCGTCCCCTCTCCGTTGAGCCCCGCGCCTTCGCCCGGGTGGGG 60
BAC_RP11866L6     TACAGTCTCACCACGCCCCGTCCCCTCTCCGTTGAGCCCCGCGCCTTCGCCCGGGTGGGG 60
                  ************************************************************

HDiPS_Short       CGCTGCGCTGTCAGCGGCCTTGCTGTGTGAGGCAGAACCTGCGGGGGCAGGGGCGGGCTG 120
HDiPS_Long        CGCTGCGCTGTCAGCGGCCTTGCTGTGTGAGGCAGAACCTGCGGGGGCAGGGGCGGGCTG 120
C127              CGCTGCGCTGTCAGCGGCCTTGCTGTGTGAGGCAGAACCTGCGGGGGCAGGGGCGGGCTG 120
C116              CGCTGCGCTGTCAGCGGCCTTGCTGTGTGAGGCAGAACCTGCGGGGGCAGGGGCGGGCTG 120
BAC_RP11866L6     CGCTGCGCTGTCAGCGGCCTTGCTGTGTGAGGCAGAACCTGCGGGGGCAGGGGCGGGCTG 120
                  ************************************************************

HDiPS_Short       GTTCCCTGGCCAGCCATTGGCAGAGTCCGCAGGCTAGGGCTGTCAATCATGCTGGCCGGC 180
HDiPS_Long        GTTCCCTGGCCAGCCATTGGCAGAGTCCGCAGGCTAGGGCTGTCAATCATGCTGGCCGGC 180
C127              GTTCCCTGGCCAGCCATTGGCAGAGTCCGCAGGCTAGGGCTGTCAATCATGCTGGCCGGC 180
C116              GTTCCCTGGCCAGCCATTGGCAGAGTCCGCAGGCTAGGGCTGTCAATCATGCTGGCCGGC 180
BAC_RP11866L6     GTTCCCTGGCCAGCCATTGGCAGAGTCCGCAGGCTAGGGCTGTCAATCATGCTGGCCGGC 180
                  ************************************************************

HDiPS_Short       GTGGCCCCGCCTCCGCCGGCGCGGCCCCGCCTCCGCCGGCGCAGCGTCTGGGACGCAAGG 240
HDiPS_Long        GTGGCCCCGCCTCCGCCGGCGCGGCCCCGCCTCCGCCGGCGCAGCGTCTGGGACGCAAGG 240
C127              GTGGCCCCGCCTCCGCCGGCGCGGCCCCGCCTCCGCCGGCGCAGCGTCTGGGACGCAAGG 240
C116              GTGGCCCCGCCTCCGCCGGCGCGGCCCCGCCTCCGCCGGCGCAGCGTCTGGGACGCAAGG 240
BAC_RP11866L6     GTGGCCCCGCCTCCGCCGGCGCGGCCCCGCCTCCGCCGGCGCAGCGTCTGGGACGCAAGG 240
                  ************************************************************

HDiPS_Short       CGCCGTGGGGGCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACC 300
HDiPS_Long        CGCCGTGGGGGCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACC 300
C127              CGCCGTGGGGGCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACC 300
C116              CGCCGTGGGGGCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACC 300
BAC_RP11866L6     CGCCGTGGGGGCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACC 300
                  ************************************************************

HDiPS_Short       TGCGGCCCAGAGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGG 360
HDiPS_Long        TGCGGCCCAGAGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGG 360
C127              TGCGGCCCAGAGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGG 360
C116              TGCGGCCCAGAGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGG 360
BAC_RP11866L6     TGCGGCCCAGAGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGG 360
                  ************************************************************

HDiPS_Short       CCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGA 420
HDiPS_Long        CCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGA 420
C127              CCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGA 420
C116              CCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGA 420
BAC_RP11866L6     CCTCCGGGGACTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGA 420
                  ************************************************************

HDiPS_Short       AGCAGCAGCAGCAGCAGCA---------------------------------------- 499  - HD-iPS Short Allele (19 CAG)
HDiPS_Long        AGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC 540  - HD-iPS Long Allele (73 CAG)
C127              AGCAGCAGCAGCAGCAGCAACA------------------------------------ 502  - C127 (20 CAG)
C116              AGCAGCAGCAGCAGCAGCAGCA------------------------------------ 505  - C116 (21 CAG)
BAC_RP11866L6     AGCAGCAGCAGCAGCAGCAGCA------------------------------------ 505  - BAC(RP11-866L6) (21 CAG)
                  ******************

HDiPS_Short       ----------------------------------------------------------
HDiPS_Long        AGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC 600
C127              ----------------------------------------------------------
C116              ----------------------------------------------------------
BAC_RP11866L6     ----------------------------------------------------------

HDiPS_Short       ----------------------------------------------------------
HDiPS_Long        AGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC 660
C127              ----------------------------------------------------------
C116              ----------------------------------------------------------
BAC_RP11866L6     ----------------------------------------------------------

HDiPS_Short       -ACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCGC 558
HDiPS_Long        AACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCGC 720
C127              -GCAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCGC 561
C116              -ACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCGC 564
BAC_RP11866L6     -ACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCGC 564
                   ************************************************************

HDiPS_Short       AGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCG 618
HDiPS_Long        AGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCG 780
C127              AGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCG 621
C116              AGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCG 624
BAC_RP11866L6     AGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCG 624
                  ************************************************************

HDiPS_Short       GCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGTTTGGGCCCGCTGCAGCTCCC 678
HDiPS_Long        GCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGTTTGGGCCCGCTGCAGCTCCC 840
C127              GCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGTTTGGGCCCGCTGCAGCTCCC 681
C116              GCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGTT------------------- 665
BAC_RP11866L6     GCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACCGTGAGTTTGGGCCCGCTGCAGCTCCC 684
                  ***************************************
```

Figure S2

A


H2
H5
H4
H1
C7
C8
C1
C6
C3
H6
C5
H7
C2
C4
H3
H8

B

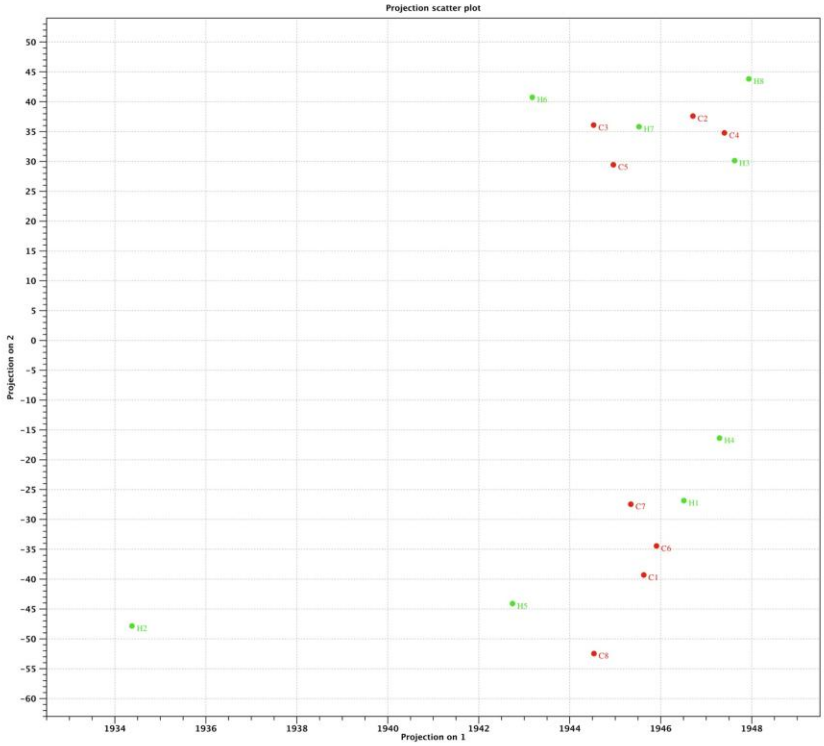

Projection scatter plot
Projection on 2
Projection on 1
1934
1936
1938
1940
1942
1944
1946
1948
H2
H5
H6
C3
H7
C2
C4
C5
H3
H8
H4
C7
H1
C6
C1
C8

Figure S3

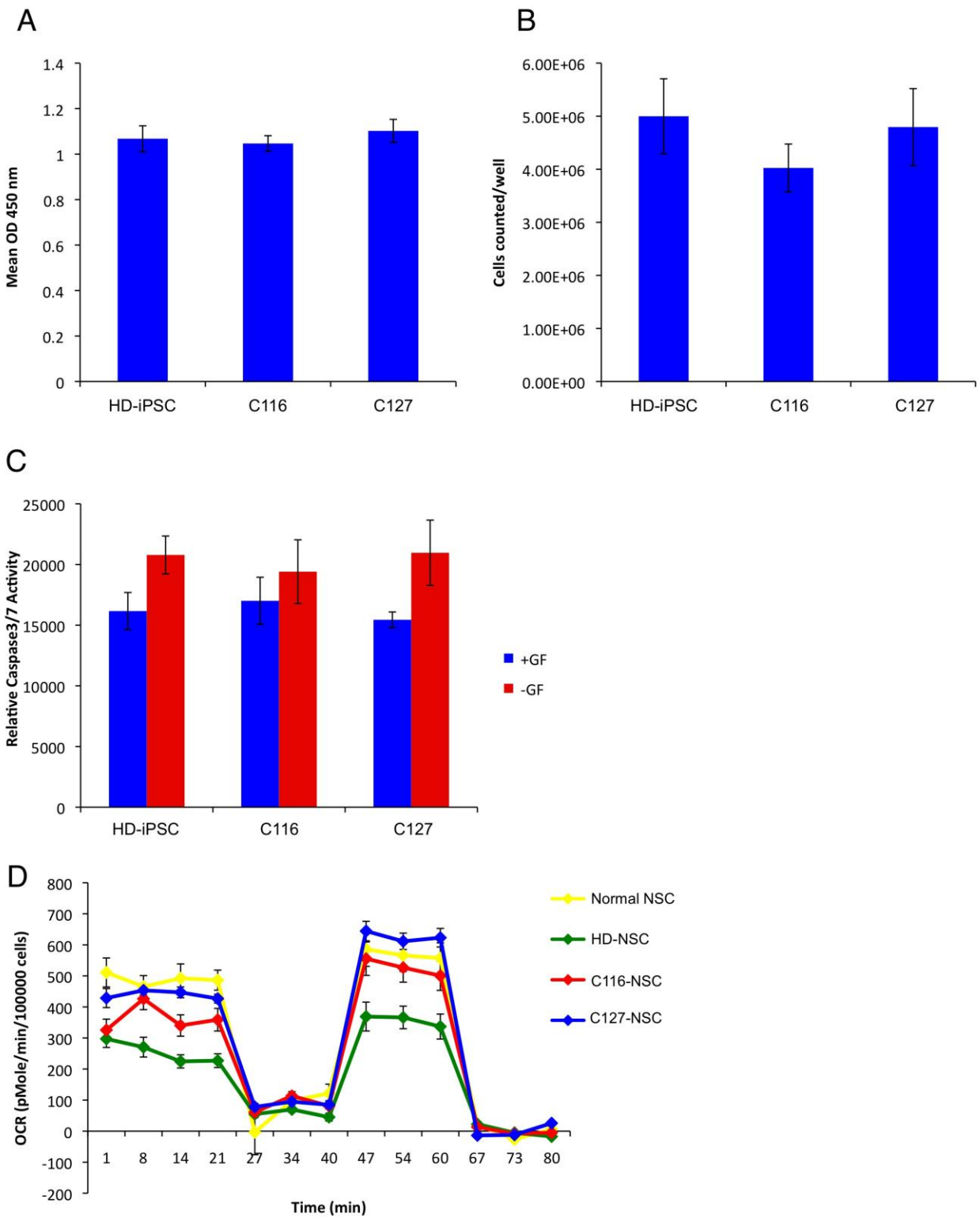
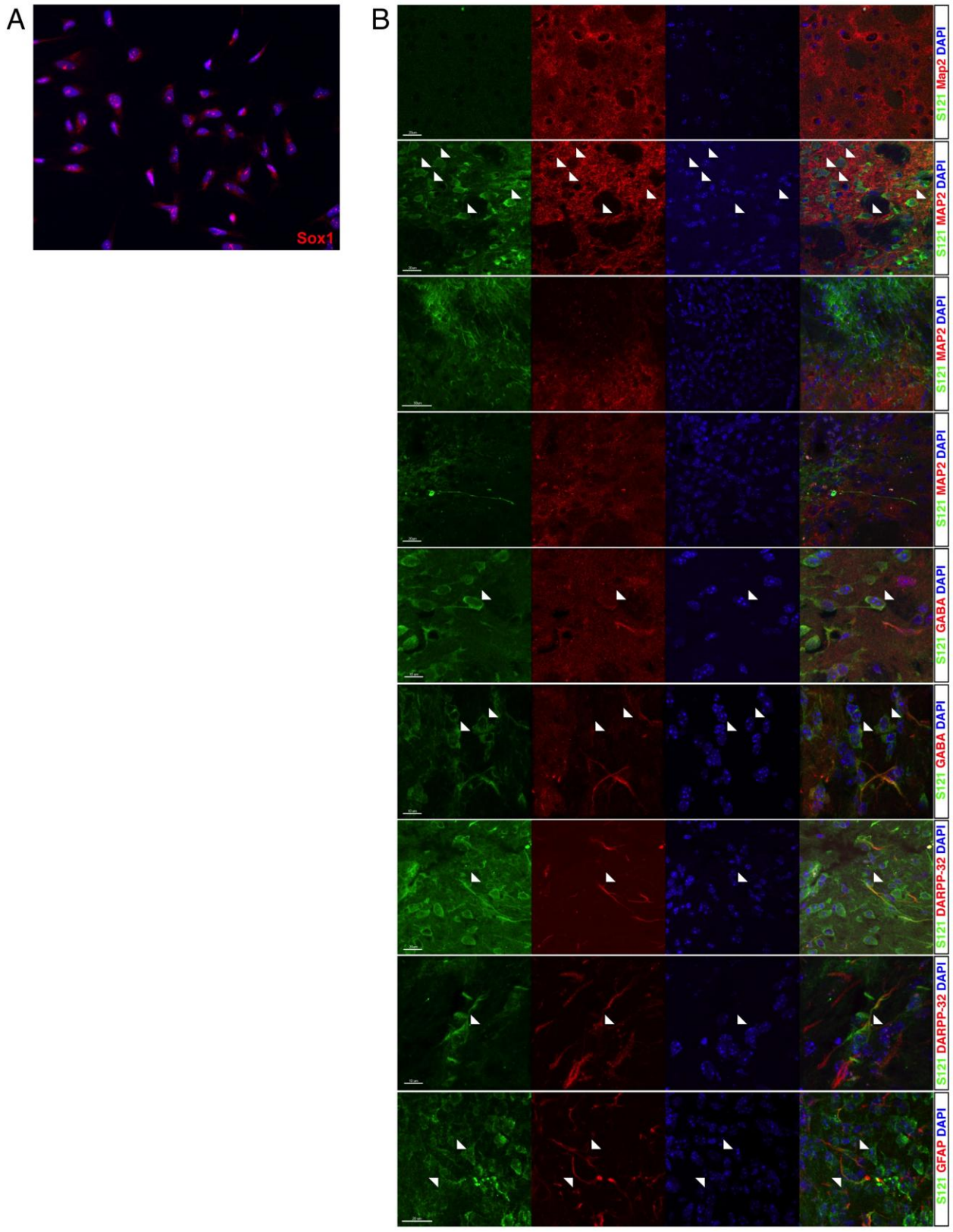

A
Mean OD 450 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HD-iPSC
C116
C127
B
Cells counted/well
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
HD-iPSC
C116
C127
C
Relative Caspase3/7 Activity
25000
20000
15000
10000
5000
0
HD-iPSC
C116
C127
+GF
-GF
D
OCR (pMole/min/100000 cells)
800
700
600
500
400
300
200
100
0
-100
-200
1
8
14
21
27
34
40
47
54
60
67
73
80
Time (min)
Normal NSC
HD-NSC
C116-NSC
C127-NSC

Figure S4


A
Sox1
B
S121 Map2 DAPI
S121 MAP2 DAPI
S121 MAP2 DAPI
S121 MAP2 DAPI
S121 GABA DAPI
S121 GABA DAPI
S121 DARPP-32 DAPI
S121 DARPP-32 DAPI
S121 GFAP DAPI

Supplemental Figures

Figure S1 (related to Figure 1). Additional validation of corrected HD-iPSC lines by southern blot analysis and sequencing

(A) Southern blot verifies absence of off-targeted vectors in clones 127 and 116. Southern blot with iPSC clone genomic DNA or BAC DNA digested with XhoI restriction enzyme results in an expected 7.5 kb band when probed with a fragment inside the neomycin cassette. Variable fragment size due to one XhoI site that is external to the targeting vector tests for off-targeted vectors. Clones 127 and 116 show a single correctly targeted band. Clone 111 shows the presence of several larger fragments suggesting incorrect targeting of the neomycin cassette in this clone.

(B) Sequencing alignment of iPSC clones for exon 1. Sequencing of individual alleles from HD-iPSC, C116, C127 and BAC RP11-866L6 in the region of exon 1 shows CAG repeat lengths of the original long and short alleles for HD-iPSC are 73 and 19 repeats. Sequence for C116 and C127 show the CAG lengths of the gel-purified longer alleles for these clones are 21 and 20 repeats respectively.

Figure S2 (related to Figure 2). Microarray analysis of HD-iPSC vs. corrected iPSC demonstrating similarity

(A) Hierarchical clustering of samples demonstrating similarity. Hierarchical clustering of the samples used in the experiment were clustered using a single linkage and Manhattan distance within CLC Genomics Workbench. The samples

appear to be relatively uniform in the gene expression space, and do not separate into their respective genotypes.

(B) Principal components analysis (PCA) of all samples used in the gene expression profiling. A projection scatter plot in 2-D across the first and second largest components of variability within all samples used in the gene expression profiling reveals no clustering of samples by genotype, indicating relative uniformity of gene expression across all samples.

Figure S3 (related to Figure 3). Further characterization of the HD-iPSC and corrected iPSCs

(A) Correction of HD-iPSC does not alter rate of iPSC proliferation. Cell proliferation was assayed in HD-iPSC, C116, and C127 equally seeded in 96 well plates using a BrdU cell proliferation assay kit. BrdU incorporation is measured HRP substrate measured by absorbance (Mean OD 450 nm).

(B) Relative cell proliferation of iPSC lines quantified by average cells counted/well 3 days after equal plating of HD-iPSC, C116, and C127

(C) Polyglutamine expansion does not cause additional elevated caspase activity after serum/growth factor starvation in undifferentiated iPSCs. HD-iPSC and corrected iPSC (C116 and C127) were seeded at normal conditions. Starved cells all received a 24h serum/growth factor free condition (-) while control cells were in normal medium (+). Caspase 3/7 activity assay was carried out. Unlike the situation of differentiated NSCs, HD-iPSCs did not show higher caspase activity after starvation compared to C116 or C127.

(D) NSCs derived from corrected and normal iPSC show higher maximum respiration capacity than NSCs from uncorrected HD iPS cells. Corrected NSCs (C116-NSCs and C127-NSCs), normal NSCs and uncorrected NSCs (HD-NSCs) were seeded with normal conditions. After 72h cells were analyzed on a Seahorse XF24 Extracellular Flux Analyzer for oxygen consumption rate (OCR) with sequential additional of drugs. Oligomycin (an ATP synthase inhibitor) will shut down mitochondria respiration while FCCP (an uncoupler) will make mitochondria work at highest speed and OCR at this time reflects mitochondria maximum respiration capacity. Finally rotenone and antimycin-A will destroy mitochondria electron transfer chain completely to show background. After background reduction (subtraction of OCR after rotenone/antimycin-A) and normalization (against cell number), corrected and normal NSCs showed higher maximum respiration capacity than uncorrected NSCs.

Figure S4 (related to Figure 4). ***In vitro*** **and** ***in vivo*** **differentiation of corrected NSCs**

(A) NSCs were derived from corrected HD-iPSCs with EB method and stained for NSC marker Sox1, with counter staining for DAPI.

(B) Immunofluorescence of R6/2 mouse brain sections two weeks after NSC striatal injection of corrected NSCs (C116-NSCs). Markers detecting human specific STEM121 (S121) and either MAP2, GABA, DARPP-32, GFAP are shown as individal panels with counter staining for DAPI. The first row of images shows STEM121 staining control outside of the injection region.

Supplemental Methods

Culture and nucleofection of HD-iPSCs

When grown on MEFs, the ES culture medium was knockout DMEM/F12 supplemented with 20% knockout serum replacement, 2.48 mM L-glutamine, 1X nonessential amino acid, 15.4 mM HEPES, 50 μM β-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin (Cellgro) and 4 ng/ml bFGF (Peprotech). When grown on Matrigel, the ES medium conditioned by MEFs was used. The HD-iPSCs were passaged with 1 mg/ml collagenase. Before nucleofection iPSCs grown on matrigel were passaged twice by TrypLE express treatment for 45-60 seconds. For nucleofection, a Human Stem Cell Nucleofectior kit 1 (Lonza) was used. Cells grown to 70-80% confluency were pre-treated with 10 μM Y-27632 ROCK inhibitor for 1 hr. Cells were dissociated by TrypLE treatment for 1.5-2 min and spun down at 200 g for 2.5 min. 1 x 10^6 cells were resuspended in 100 μl Nucleofector solution including supplement and immediately nucleofected with program A-27. Cells were transferred to 2 ml prewarmed RPMI+20% KSR and incubated at 37°C for 15 min, then transferred to prewarmed conditioned medium on matrigel coated plates at a density of 1-2 x 10^6 cells per 10 cm^2. G418 at 50 μg/ml was added starting at 24 hours post-nucleofection and continued for 3 weeks with daily changes of conditioned medium. Surviving colonies at 3 weeks were manually passaged by incubation with 0.4 mg/ml collagenase for 30-90 minutes, dissociation with a 200 μl pipette tip, and transfer to 96-well plates.

PCR screen and southern blot analysis

Duplicates of iPSC clones were grown and passaged to 24-well plates. Cells were incubated overnight in lysis buffer, followed by precipitation in equal volume of isopropanol and 70% EtOH wash. DNA was resuspended in TE. The crude genomic preparation was screened first by PCR amplifying the CAG repeat region of the *HTT* gene. Clones showing the loss of the expanded allele were tested by southern blot analysis. Genomic DNA was further purified by phenol/chloroform extraction, EtOH precipitation, and 70% EtOH wash. 15 μg of the purified genomic DNA were digested by HindIII, followed by a second phenol/chloroform and precipitation. Digested DNA was run on 0.7% agarose gel, transferred to Hybond N+ nylon membranes, and crosslinked by stratalinker. A 300 bp fragment external to the short arm of the targeting construct and designed to produce 10 kb and 5 kb hybridizing HindIII Southern bands for the wild type and targeted allele respectively was radiolabeled by P^{32} using a DECAprime II kit (Ambion) and unincorporated nucleotides were purified out by NucAway Spin columns (Ambion). Labeled probe was hybridized with blots in ExpressHyb solution (Clontech) overnight at 68°C. Blots were washed in 0.2x SSC, 0.1% SDS at 66°C until background signal was removed. Labeled blots were imaged on film.

Of significance to screening iPSCs, we observed a slow and low percentage recovery from frozen stocks compared to mouse ES cells. Therefore, standard mouse ES based large scale cryopreservation methods were not feasible for use during our screenings. Therefore, it was of practical importance to reduce the active and daily

fed screening population as quick as possible. Once the pool of PCR positive candidates was screened and verified by a second PCR, the remaining candidates were discarded with confidence and individual candidate clones were expanded and frozen down using standard protocols, while southern screening was performed. Due to the nature of the stepwise approach our screen does not account for clones in which targeting and recombination occurred on the normal (unexpanded) allele leaving the expanded *HTT* allele intact, as these would score as negative on the primary CAG-based PCR screen. Because we wanted to correct the expanded allele, we felt it unnecessary to pursue these clones, however for comparison to previously reported gene targeting frequencies, the expected full homologous recombination frequency would range to roughly double what we report for the expanded allele given equal affinity for recombination on both alleles. This is comparable to previous reported frequencies of gene targeting in human stem cells (Costa et al., 2007; Song et al., 2010; Zwaka and Thomson, 2003).

Neuronal differentiation of HD-iPSCs

iPSCs were passaged with collagenase and cell clumps were cultured in a low attachment petri-dish (Kord-Valmark) in ES medium without bFGF. Medium was replaced every 2 days. Each medium change 25% more ES medium was replaced by EB differentiation medium (DMEM supplemented with 20% fetal bovine serum, 1X nonessential amino acid, 50 μM β-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin). After 8 days the medium was 100% EB differentiation medium. For verification of three germ layers differentiation potential, EBs were

attached to Matrigel coated surface in FBS containing EB differentiation medium for 2 weeks before ICC. For neural differentiation poly-ornithine/laminin (pO/L) coated plates were prepared by incubating culture plates with 40 μg/ml poly-ornithine (Sigma) for 1h at 37°C followed by 10μg/ml laminin (Sigma) for 1h at 37°C. After 10 days the EBs in suspension were attached onto pO/L coated plates in neural differentiation medium (DMEM/F12 supplemented with 1X N2, 100 U/ml penicillin and 100 μg/ml streptomycin) with addition of 25 ng/ml bFGF. Medium was replaced every 2 days. After 10-12 days rosettes were manually picked, triturated by P1000 tip and plated on pO/L coated plates in neural proliferation medium (Neurobasal medium supplemented with 1X B27, 2mM L-Glutamine, 25 ng/ml bFGF, 10 ng/ml LIF, 100 U/ml penicillin and 100 μg/ml streptomycin). We used the ENStem-A neural expansion medium (Millipore) which is Neurobasal medium containing B27 and LIF as the basis to make our own neural proliferation medium. NSCs were passaged with Accutase (Sigma). For purposes other than striatal neuron differentiation, NSCs were grown on Matrigel after the first several passages on pO/L. The striatal differentiation of HD-NSCs was induced by changing neural proliferation medium to neural differentiation medium (without bFGF) supplemented with 250 ng/ml SHH (R&D Systems), 100 ng/ml DKK1 (R&D Systems), 20 ng/ml BDNF (Peprotech) and 10 μM Y27632 (Calbiochem). After 19 days in the condition above, cells were exposed to 0.5 mM dibutryl-cyclic AMP (Sigma), 0.5 μM valpromide (Alfa Aesar), 20 ng/ml BDNF, 10 μM all-trans retinoic acid (RA, from Sigma) and 10 μM Y27632 for an additional 3 days to induce DARPP-32 positive striatal neurons.

Immunocytochemical staining

Cells grown on cover slips were fixed with 4% paraformaldehyde (Sigma) for 20 min at room temperature (RT), then permeabilized with 0.1% Triton X-100 (Sigma) for 10 min and blocked with 5% donkey serum (Millipore) for 45 min. Incubation in primary antibodies (PBS with 1% bovine serum albumin, BSA, from Roche) was overnight at 4°C followed by three washes with PBS, then with Alexa conjugated secondary antibodies (Invitrogen) in PBS with 1% BSA for a 1.5 h incubation at RT. The cells were washed with PBS for 3X and mounted to a slide with droplets of Prolong Gold with DAPI (Invitrogen). The following primary antibodies were used: Nanog, Oct3/4, Sox2, SSEA-4 (the above 4 are all from R&D Systems), TRA-1-60 (Stemgent), Nestin (Abcam), Sox17 (R&D systems), SMA (Abcam), Sox1 (Abcam), DARPP-32 (Cell Signaling), Calbindin (Sigma), GABA (Millipore) and β-III-Tubulin (Sigma).

Western blot analysis

HD-iPSCs, normal iPSCs and corrected HD-iPSCs were detached by 1 mg/ml collagenase (Invitrogen) incubation at 37°C for 1h. NSCs were detached by Accutase. Cells were briefly centrifuged and washed once with DPBS (Cellgro). Cell pellets were lysed by sonication in mammalian protein extraction reagent (M-PER, from Thermo Scientific) containing protease inhibitors (one Complete mini tablet per 10

ml, Roche) and 1% phosphatase inhibitor cocktail set II (Calbiochem). Protein concentration was measured with Pierce BCA protein assay kit (Thermo Scientific) to ensure equal sample loading. Protein samples were run on 3-8% tris-acetate gel (Invitrogen) for iPSC samples or on 4-12% bis-tris gel (invitrogen) for NSCs, transferred to nitrocellulose membrane (Whatman), probed with appropriate primary antibodies overnight at 4°C. Anti-huntingtin (1:1000, MAB2166, Millipore) and anti-polyglutamine (1:1000, 1C2, Millipore), N-cadherin (1:250, BD Biosciences) and GAPDH (1:10,000, Fitzgerald). HRP conjugated secondary antibodies (GE Healthcare) were added for 1 h at RT followed by incubation with ECL reagent (Thermo Scientific) for 1 min at RT. The blot was developed with Amersham Hyperfilm (GE) and processed with a 100Plus Automatic X-ray Film Processor (All-Pro Imaging).

Gene arrays

RNA yield and integrity were then evaluated via Bioanalyzer (Agilent, Santa Clara, CA, USA) All samples presented in this report passed QC and integrity checks, and were then reverse transcribed and amplified with one round of whole-transcriptome amplification via the WTA2 complete whole transcriptome kit from Sigma (Sigma-Aldrich, St Louis, MO, USA). After amplification, the resulting omniplex cDNA library was then further purified using a Qiagen PCR purification chip (Qiagen), and evaluated for integrity and yield via Bioanalyzer. The resulting cDNA libraries were then labeled via the one color Nimblegen labeling kit as per the manufacturers instructions (Roche Nimblegen, Madison, WI, USA), and hybridized

onto 12-plex 135K (HG18) Nimblegen oligonucleotide whole genome microarrays (Roche). The hybridized arrays were washed, scanned and quantitated as per the manufacturers instructions via a 4-bay MAUI hybridization unit (Roche), and a GenePix 4200 Scanner (Molecular Devices, USA). The quantitated images were used to evaluate relative gene expression between the transformed cell lines versus controls. Normalization and statistical tests using a 5% false discovery rate (Bejamini-Hochberg) for differential expression between the transformed and control lines was carried out within ArrayStar (DNAStar, Madison, WI, USA), as well as hierarchical clustering, and principal components analysis to determine sample grouping.

Enrichment analysis of differentially expressed genes

In order to understand the functions of the differentially expressed genes between our initial cells and the genetically corrected HD-iPSCs we searched for statistically overrepresented pathways (KEGG (Kanehisa and Goto, 2000) or Reactome (Croft et al., 2011)) or Interpro (Hunter et al., 2009) protein domains. As a first step in this analysis we divided the set of genes into two groups, depending on whether the gene expression is significantly higher or significantly lower in the genetically corrected HD-iPSCs when compared to our starting cells. Subsequently we utilized DAVID, a web application that performs term enrichment on a variety of different gene annotation sources. DAVID (Huang da et al., 2009) applies a hypergeometric test to identify whether a concept (e.g. one pathway or one specific domain) is

statistically significantly more observed in our genes than expected by chance. To correct for falsely reported concepts due to testing of multiple hypothesis, we adjusted the calculated p-value using the Benjamini-Hochberg correction. As a background for this analysis we selected all genes on the microarray. With a threshold on the adjusted p-value of 0.1 we detected that the Interpro protein domains "IPR013164:Cadherin, N-terminal", "IPR002126:Cadherin", and "IPR002190:MAGE protein" were overrepresented in genes that have a lower expression level in the HD-iPSCs, and the KEGG pathway "hsa04350:TGF-beta signaling pathway" and Reactome pathway "REACT_604:Hemostasis" were statistically enriched in genes with a higher expression level in HD-iPSCs.

RT-qPCR

Total RNA was purified from iPSCs or NSCs using RNeasy Mini kit (Qiagen) after growth on matrigel coated plates and conditioned medium containing 8 ng/ml bFGF. A minimum of 2 passages growth in tandem was established prior to collection of mRNA for comparative analysis. 1μg of RNA was converted to cDNA by using the Message Sensor RT kit (Applied Biosystems). Real time quantitative PCR (qPCR) was performed with Universal Probe Library dye (UPL from Roche) on the LightCycler 480 system (Roche). For quantification the threshold cycle Cp of each amplification was determined by the 2nd derivative analysis provided by the LightCycler 480 software and the $2^{-\Delta\Delta Cp}$ method was used to determine the relative expression level of each gene normalized against the house-keeping gene β-actin

(ActB). The qPCR primers are: *PCDH11* Forward 5'-CAGAGAACTCGGCTATAAACTCTAAAT-3', Reverse 5'-GGCCAAAAATGTTTTGACTCTT-3', *PCDHGB13* Forward 5'-GGATGCCAAAGGGAGGAC-3', Reverse 5'-ATCTGAGGCTGTGGTTATTCTACA-3'; *PCDHGA10* Forward 5'-ATTTGCCTGTGGGCACTC-3', Reverse 5'-TCACTTCTCCATTGGCACCT-3'; *PCDHGA2* Forward 5'-TGCAGACGTAGGTGAGAACG-3', Reverse 5'-CTTGTTCCCATCAGCTCCTC-3'; *CDKN2B* Forward 5'-GCGGGGACTAGTGGAGAAG-3', Reverse 5'-CTGCCCATCATCATGACCT-3'; *ID2* Forward 5'-ATATCAGCATCCTGTCCTTGC-3', Reverse 5'-AAAGAAATCATGAACACCGCTTA-3'; *ID4* Forward 5'-TTGGCCTGGCTCTTAATTTG-3', Reverse 5'-TGCAATCATGCAAGACCACT-3'; *LEFTY2* Forward 5'-AAAGAGGTTCAGCCAGAGCTT-3', Reverse 5'-CACCAGCAGGTGTGTGCT-3'; *PITX2* Forward 5'-CCTTACGGAAGCCCGAGT-3', Reverse 5'-CCGAAGCCATTCTTGCATA
-3'; *THBS1* Forward 5'-CAATGCCACAGTTCCTGATG-3', Reverse 5'-TGGAGACCAGCCATCGTC-3'; *BDNF* Forward 5'-GTAACGGCGGCAGACAAA-3', Reverse 5'-GACCTTTTCAAGGACTGTGACC-3'; *TGF beta 1* Forward 5'-GCAGCACGTGGAGCTGTA-3', Reverse 5'-CAGCCGGTTGCTGAGGTA-3'; *N-cadherin* Forward 5'-CTCCATGTGCCGGATAGC-3', Reverse 5'-CGATTTCACCAGAAGCCTCTAC-3'; *ActB* Forward 5'-CCAACCGCGAGAAGATGA-3', Reverse 5'-CCAGAGGCGTACAGGGATAG-3'.

BrdU proliferation assay and cell counting

BrdU Cell Proliferation Assay Kit (96 well format) (Cell Signaling Technology) was used to measure proliferation of iPSC lines. Cells were seeded equally in 96-well format, and after 3 days growth in normal iPSC medium, fixed and stained with antibody for BrdU. HRP-linked antibody and HRP substrate were used to detect level of incorporation by an absorbance measurement on 96 well plate reader. Relative BrdU incorporation was quantified by average OD reading (n=8). Cells were also counted (n=8) 3 days after equal seeding using a TC10 automated cell counter (Bio-rad).

Caspase-3/7 activity assay

NSCs or iPSCs were grown in 24-well plates. For starved samples cells were first washed once with neural proliferation medium without LIF and bFGF (for NSCs) or ES medium without KSR and bFGF (for iPSCs), then cultured in this growth factor/serum free medium for 24 h. After 24 h of growth factor/serum withdrawal, medium was removed and 150 µl 1X lysis buffer (1:1 mixture of Apo3 HTS lysis buffer with DPBS) was added into each well. The plates were rotated on an orbital shaker at 700 rpm for 10 min. After that 30 µl of cell lysate was dispensed into one well of a 96 well plate in triplicate for each sample. 70 µl of substrate mix (1X lysis buffer with 1X Apo3 HTS Caspase3/7 detection reagent and 20 mM DTT) was added into each well and the plate was shaken at 700 rpm for 30 s. Then the plate was loaded on Fusion-Alpha Universal Microplate Analyzer (Perkin Elmer) for the fluorescence based reading (Ex: 485nm, Em: 530nm). For each sample 10 µl of

lysate was dispensed into another 96-well plate in triplicate for protein concentration measurement with Pierce BCA protein assay kit. The caspase activity was normalized against protein concentration for each sample.

TUNEL assay

TUNEL assay was performed with the DeadEnd Fluorometric TUNEL System (Promega). Normal NSCs, corrected NSCs (C116-NSCs and C127-NSCs) and uncorrected HD-NSCs were cultured on 8-well chamber slide (Nunc) in normal condition for 2 days. Then the cells were washed once with NeuroBasal medium (Invitrogen) without any additive. After wash cells were kept in this growth factor withdrawal medium for 24 h before fixation with 4% paraformaldehyde (Sigma) for 20 min. After wash with DPBS (Cellgro) cells were permeablized with 0.2% Triton X-100 (Sigma) for 5 min, equilibrated with equilibration buffer for 10 min at RT and incubated with a reaction mixture containing fluorescein-12-dUTP and recombinant terminal deoxynucleotidyl transferase (rTdT) for 1h at 37^{o}C. After the reaction cells were incubated in 2XSSC for 15min at RT and mounted in Prolong Gold with DAPI (Invitrogen). Reagents are all provided by the Promega kit unless otherwise indicated.

Respirometry analysis

The respirometry analysis of HD and corrected NSCs was carried out using an XF24 extracellular flux analyzer (Seahorse Bioscience). Both HD-NSCs and corrected C116-NSCs were seeded in an XF24 cell culture microplate with standard culture

conditions. Three days after seeding, right before respirometry analysis, cells were washed three times with “Seahorse buffer” (120 mM NaCl, 3.5 mM KCl, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 20 mM TES, 1.2 mM Na_2SO_4, 2 mM $MgCl_2$, 15 mM glucose, 15 mM sodium pyruvate, pH 7.4) and cells were left in “Seahorse buffer” at 37^{o}C. The plate was loaded into the XF24 analyzer for a program with 13 cycles of 1 min mix, 2 min wait and 3 min measurement. 2 μg/ml oligomycin was added into cells after the 4th cycle. 1 μM FCCP was added after the 7th cycle. 2 μM rotenone and 1 μM antimycin A were added after the 10th cycle. The oxygen consumption was shown as pMole O_2/min after normalization against cell density.